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A STUDY OF THE INSULIN-INACTIVATING SUBSTANCES OF THE LIVER

[Following is a translation of an article by P'ei Yuan-ching (裴元靜), Nan Kuo-chu (南國柱), Liu Li-ch'un (刘立春), Peiping Hospital, Peiping, in Sheng-li Hsueh-pao (Journal of Physiology), June 1960, Vol 24, No 2, pp 136-140.]

Not a few reports have been published on studies of the substances in the liver that inactivate insulin. In general normal animals were used in those studies. The livers of the animals were ground up and extracts from the ground tissues were transferred to test tubes for observation of any change in the characteristics of the insulin. 1,2/ In some cases the extracts were subjected to insulin-iodine analyses for determination of the effect of the inactivating substances on the insulin. 3,4/ This paper reports our studies on the action of insulin-inactivating substances in animals under anesthesia by perfusing the liver in situ, and on our inquiry into what changes inactivating substances underwent when tolbutamide rendered the animals hypoglycemic.

Materials and Procedure

Kinds of Animals Used

Large white rats--all were mature and healthy, both male and female, each weighing 120-150 grams. Feeding was dispensed with the night before the experiment. These animals were used for liver perfusion experiments.

Rabbits--all were mature and healthy, both male and female, each weighing 1.5 kilograms or more. These animals were used for experiments to determine the activity of insulin.

Feeding was dispensed with the night before the experiment. In the morning of the day when the experiment was to take place, each rabbit was given a blood sugar content test five times at half-hour intervals when its stomach was without food. Those with comparatively stable sugar content of blood were picked for experimentation.

Chemicals Used

Insulin--Insulin Roxane (made in the Netherlands). Label declared 40 international units per ml; released May 11, 1957, good until May 11, 1959.

Locke's Solution--NaCl 0.9%, KCl 0.04%, $NaHCO_3$ 0.05%, $CaCl_2$ 0.018%.

Procedure

(1) Liver Perfusion in Large White Rats. As anesthesia, 1 gm of a 25% solution of ethyl carbamate (urethane) was injected into the abdominal cavity of the animal. When the animal was anesthetized and its body temperature registered about 37°C a horizontal incision was made on its abdomen. The liver was carefully raised and a catheter was inserted into the portal vein, so that the Locke's solution could enter the liver thru the catheter. Similarly another catheter was inserted into the vena cava inferior through which the perfusate was to come out and be collected. Then the bile duct, the hepatic artery and the upper portion of the portal vein above the septum were tied, so that the circulatory system of the liver was completely isolated from the rest of the body. During the operation extraordinary care was exercised so that the liver would not be injured and perfusion would not fail. The fluid pressure of the Locke's solution used for the perfusion was 25-30 mm. A regulating clip with a screw for adjustment was employed to maintain the flow of perfusion at 0.2-0.4 ml per minute. The original position of the liver was strictly maintained. Difficulties will develop in the perfusate outflow when the position of liver is changed during the operation.

(2) Handling of the Various Kinds of Fluids for the Experiment.

(a) Preparation of blood fluid prior to perfusion--Prior to the process of perfusion, some blood was collected from the vena cava inferior with which approximately 10 ml Locke's solution was made. To 20 international units of Insulin Roxane we added Locke's solution until the mixture measured 10 ml (each ml of the mixture thus contains

2.0 international units of Insulin Roxane). Observation was made of the action of this blood fluid on the insulin kept at a constant temperature of 37°C for one hour.

(b) Perfusate--When perfusion was under way insulin was added from time to time according to the requirement of the liver perfusate for the maintenance of its insulin concentrate of 2.0 international units per ml. Similarly, observation was made of the action of the liver perfusate on the insulin for one hour at a constant temperature of 37°C.

(c) Insulin perfusion fluid-- In the process of perfusion 1 ml of insulin with a concentration of 40 international units per ml was introduced at the rate of 8 international units per minute into the rubber tubing at the entrance of the portal vein. In the meantime, the perfusate was collected at the exit, until 20 ml had been obtained. Each ml of the perfusate collected supposedly contained 2.0 international units of insulin. The perfusate collected was set aside so that the insulin would settle. To make sure that all the insulin that went in had been entirely recovered in the 20 ml perfusate collected, a separate experiment was performed in which a solution of indigocarmine with a concentration of 6 mg per ml was introduced into the portal vein in a manner similar to that stated above. It was obvious that 20 ml of the perfusate was collected and all the indigocarmine had been drained from the liver.

(d) Liver extracts--Following the perfusion the liver was removed. To the liver an equal amount in weight of Locke's solution was added to make a liver extract according to Mirsky's procedure.2/ The extract was then placed in a centrifuge to rotate at a speed of 3,500 revolutions per minute. This done, 10 ml was taken out from the substances in suspension in the top layer. To this 20 international units of insulin and 10 ml of Locke's solution were added and kept at 37°C for one hour. Observation was made of the action of the liver extract on the insulin.

(3) Observation of the activity of insulin. Determination of the normal sugar content of the blood of the rabbits picked for the experiment was made by testing the blood of the animals three times in half-hour intervals, employing the Folin-Wu method. The average of measurements was used as the normal sugar content of blood. Then the various fluids, the amount of each of which being determined by its strength being equivalent to 2.0 international units

2/ Refer to footnote 2, page 1.

were injected intravenously into two rabbits. After the injection a test was given every half an hour three times to determine the sugar content of the blood. The respective sugar content values obtained were compared with the normal sugar content value. The percentage of decrease indicated the degree of the activity of the insulin.

(4) Rendering the White Rats Hypoglycemic with Tolbutamide. After determination has been made of the sugar content of the blood when the stomach is without food, tolbutamide was administered by mouth to the animal, the dosage being 2 grams per kilogram body-weight. A test was given every hour after the administration of the tolbutamide to determine the sugar content of the blood. In general, two hours after the chemical had been introduced the sugar content dropped to the minimum, namely about 30%. This done, perfusion followed.

Results and Discussion

(1) The Effect of Fluid Collected Before and Fluid Collected After Perfusion of Liver on the Activity of the Insulin

At the conclusion of four separate experiments (see Table I) three portions of perfusion fluid were collected consecutively in the process, each portion containing 20 ml. Non of these portions of perfusion fluid caused any change in the activity of the insulin. But the extract obtained from the ground-up liver that had been perfused did inactivate insulin in test tube tests. The blood fluid collected from the vena cava inferior before persuion had no inactivating effect on the insulin.

In the light of the results of this experiment it may be said that the insulin-inactivating substance cannot be carried away from the liver by the perfusion fluid, and that it is not present in the blood fluid. Even after the liver has been washed by a large quantity of perfusion fluid, the insulin-inactivating property of the substances are still preserved in the liver.

[Table I follows.]

Table I

The Effect of (1) Perfusion Fluid Containing No Insulin and (2) Extracts Collected After Perfusion on the Activity of Insulin

Fluid Tested		Perfusion Fluid: First Portion			Perfusion Fluid: Second Portion			Perfusion Fluid: Third Portion			Extract from Liver After Perfusion			Blood Fluid from Vena Cava Inferior Before Perfusion		
Intervals (in min.) between Tests of Sugar Content of Blood of Rabbits after Injection		30	60	90	30	60	90	30	60	90	30	60	90	30	60	90
Degree of Activity of Insulin (as indicated by the decrease of sugar content of blood, expressed percentagewise)	Average Value of 4 Tests (±%)	-42	-41	-27	-38	-42	-31	-49	-47	-37	+3	+6	+8	-50	-52	-46
	Deviation (±)	3.7	5.6	7.0	6.7	7.4	8.7	2.3	7.1	8.8	4.6	5.3	14.8	2.8	5.7	7.2

(2) The Effect of Liver on the Activity of the Insulin in the Perfusion Fluid

After seven experiments (see Table II) it was found that the insulin in the perfusion fluid that had passed through the liver was inactivated in the process, as shown in the tests made of the perfusion collected which did not bring about a decrease in the sugar content of the blood of the rabbits. But when insulin was added to the perfusion fluid before and after perfusion, the former was not affected. We may infer from these phenomena that when insulin passes through the liver it is inactivated. The results of our experiment confirm the findings of Mirsky who found that when he added some extract from ground-up liver to a test tube containing insulin, the latter was rendered inactive. The fact that the insulin inactivating substances cannot be carried away from the liver by the perfusion fluid, and that they inactivate the insulin only when the latter passes through the liver tissues is a good indication that they possibly are close related to the liver tissues themselves.

Table II

Changes of the Activity of Insulin When it Passes the Liver with Liver Perfusion Fluid

Fluid Tested		1st Portion of Perfusion Fluid			Perfusion Fluid Containing Insulin			2nd Portion of Perfusion			Extract of Liver After Perfusion		
Intervals (in min.) between Injections Given to Rabbits.		30	60	90	30	60	90	30	60	90	30	60	90
Activity of Insulin (as indicated by the decrease of sugar content of blood of rabbit)	Average of 7 tests (±%)	-37	-45	-45	-7	-4	+1	-37	-40	-38	-15	-21	-9
	Deviation (±)	7.9	9.4	9.5	4.0	3.2	2.4	7.7	2.0	6.0	17.4	12.1	17.6

Besides, as the results of our experiment show, after the perfusion fluid containing insulin passed through the liver, the effect of extracts from the liver on insulin was not always the same; consequently the results of the experiment in this particular respect were comparatively inconsistent. Further observations showed that in each perfusion it was not easy for the perfusion fluid to pass through each lobe of the liver--in fact the perfusion fluid could not pass through certain parts of that organ. As a result, those parts kept their original red color in the process of perfusion. In two separate experiments (see Table III) the perfused liver was divided into two portions according to the two colors, red and pale. Observations were made of the effect of their respective extracts on the activity of insulin. The results showed that the extract obtained from the portion of the liver through which the perfusion fluid containing insulin had passed, namely the pale portion, lost its original inactivating property, while the extract from the portion that had not been penetrated by the perfusion fluid, namely the red portion, still retained its insulin-inactivating property.

Table III

The Effect of Extracts Obtained from Liver Through Which Perfusion Fluid Containing Insulin Passed

Fluid Tested		Perfusion Fluid			1st Perfusion Containing Insulin			2nd Perfusate Containing Insulin			Fluid Extracts from Liver					
											Portion					
											Red			Pale		
Intervals (in min) between tests of sugar content of blood of rabbit after injection		30	60	90	30	60	90	30	60	90	30	60	90	30	60	90
Degree of Activity of Insulin (as indicated by the decrease of sugar content of blood, expressed percentagewise).	Average of 2 tests (±%)	-33	-31	-37	+12	+6	+8	+3	+4	+5	-20	-9	+3	-48	-48	-44
	Deviation (±)	4.1	7.1	18.0	4.1	5.5	2.8	4.1	5.6	5.6	4.1	5.6	8.5	1.4	2.8	5.6

(3) The Effect of Tolbutamide on Insulin When the Former Was Adminstered to Produce Hypoglycemia

The results of four tests (see Table IV) demonstrated that when tolbutamide was administered to the large white rats to produce hypoglycemia, the insulin in the perfusion fluid lost its inactivating property upon passing through the liver. The results also showed that the perfusion fluid collected before and after insulin perfusion was made and extracts made of livers after perfusion had the same effect on insulin as perfusion fluid obtained from perfusion given normal rabbits. Based on these results, there does not seem to be any relation between the hypoglycemic action of tolbutamide and the insulin-inactivating substances of the liver.

Table IV
Effect of Liver on the Activity of Insulin After Tolbutamide Given Rats

Fluid Tested		1st Portion of Perfusion Fluid			Perfusion Fluid Containing Insulin			2nd Portion of Perfusion Fluid			Liver Extract After Perfusion		
Intervals (in min) between tests of sugar content of blood of rabbits after injection		30	60	90	30	60	90	30	60	90	30	60	90
Degree of activity of Insulin (as indicated by the decrease of sugar content of blood expressed percentagewise.)	Average of 4 tests (±%)	-36	-39	-41	-3	-1	+6	-32	-37	-41	-22	-21	-9
	Deviation (±)	3.8	7.0	6.5	3.1	5.5	2.2	6.4	7.8	10.3	23.4	21.7	20.4

In the past few years many researchers have studied again and again the relation between tolbutamide and the insulin-inactivating substances of the liver, hoping to explain the hypoglycemic mechanism of chemicals of this type [5-10]. But the results were not consistent, and the explanations attempted vary. Basing on the results of our experiment, if the hypoglycemic action of tolbutamide was caused by the insulin-inactivating substances, then when tolbutamide renders an animal hypoglycemic the activity of the insulin-inactivating substances in the liver should be decreased, and after the insulin has passed through the liver its activity should be sustained. But Table IV clearly demonstrated that the insulin in the perfusate was definitely inactivated. These results are consistent with the results that Williams[10] obtained in his observations of the reaction of the liver extract from animals that he had injected tolbutamide into.

Conclusion

In our study of the action of the insulin-inactivating substances of the livers of large white rats by perfusing the liver in situ, when a certain amount of insulin passed through the liver, it might lose its activity. This proves that the liver has an inactivating action on insulin, and that these insulin-inactivating substances are closely related to the liver tissues. Such substances could not be carried away from the liver by the perfusate. After the insulin passed through the liver, the action of the insulin-inactivating substances of the liver was weakened.

When the animals were rendered hypoglycemic by tolbutamide, the action of the insulin-inactivating substances of the liver was the same as that of the liver of normal animals, Therefore we conclude that there is no relation between the hypoglycemic action of tolbutamide and the insulin-inactivating substances of the liver.

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